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REVIEW ARTICLE

Metabolomics of Methylphenidate and Ethylphenidate: Implications in Pharmacological and Toxicological Effects

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Abstract Methylphenidate (MPH) is primarily indicated for attention-deficit hyperactivity disorder and narcolepsy therapy. A marked individual variability in the dose–response has been observed, and therefore dosage must be titrated for optimal therapeutic effect with minimal toxicity. This variability has been claimed to be predominantly pharmacokinetic. Moreover, due to its similar pharmacodynamics to amphetamine, MPH has been abused and fatalities have been reported. This review aims to discuss metabolomics of MPH, namely by presenting all major and minor metabolites. Ritalinic acid is the main metabolite. In addition, minor pathways involving aromatic hydroxylation, microsomal oxidation and conjugation have also been reported to form the *p*-hydroxy-, oxo- and conjugated metabolites, respectively. MPH may undergo transesterification with ethanol producing ethylphenidate, which is also pharmacologically active. It is expected that knowing the metabolomics of MPH may provide further insights regarding individual contribution for MPH pharmacodynamics and toxicological effects, namely if ethanol is co-consumed.

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Key Points

Methylphenidate is primarily indicated for the highly prevalent childhood neurodevelopmental attention-deficit hyperactivity disorder.

Ritalinic acid is the main inactive urinary metabolite, but minor pathways and the formation of ethylphenidate have been reported to contribute to the toxicological profile.

The characterization of metabolomics of methylphenidate enantiomers is needed for a more efficacious therapeutic drug monitoring.

1 Introduction

Methylphenidate [MPH; *dl-threo*-methyl-2(or α)-phenyl-2(or α)-(2-piperidyl) acetate; Ritalin®] is a piperazine-substituted phenylisopropylamine psychomotor stimulant approved primarily for the treatment of attention-deficit hyperactivity disorder (ADHD) and narcolepsy of children and adolescents [1–3]. As shown in Fig. 1, MPH has two stereogenic centers and therefore can exist as four possible stereoisomers (i.e., *dl-erythro* and *dl-threo*—terms used for diastereomers with two adjacent chiral carbons with two similar groups on the same or opposite sides of the carbon chain, respectively). Originally, MPH was marketed as a mixture of two racemates, 80 % *dl-erythro* and 20 % *dl-threo*. Subsequent pharmacological evaluation of the separated racemates revealed that the central stimulant activity resides only in the *threo* racemate [4], whereas both

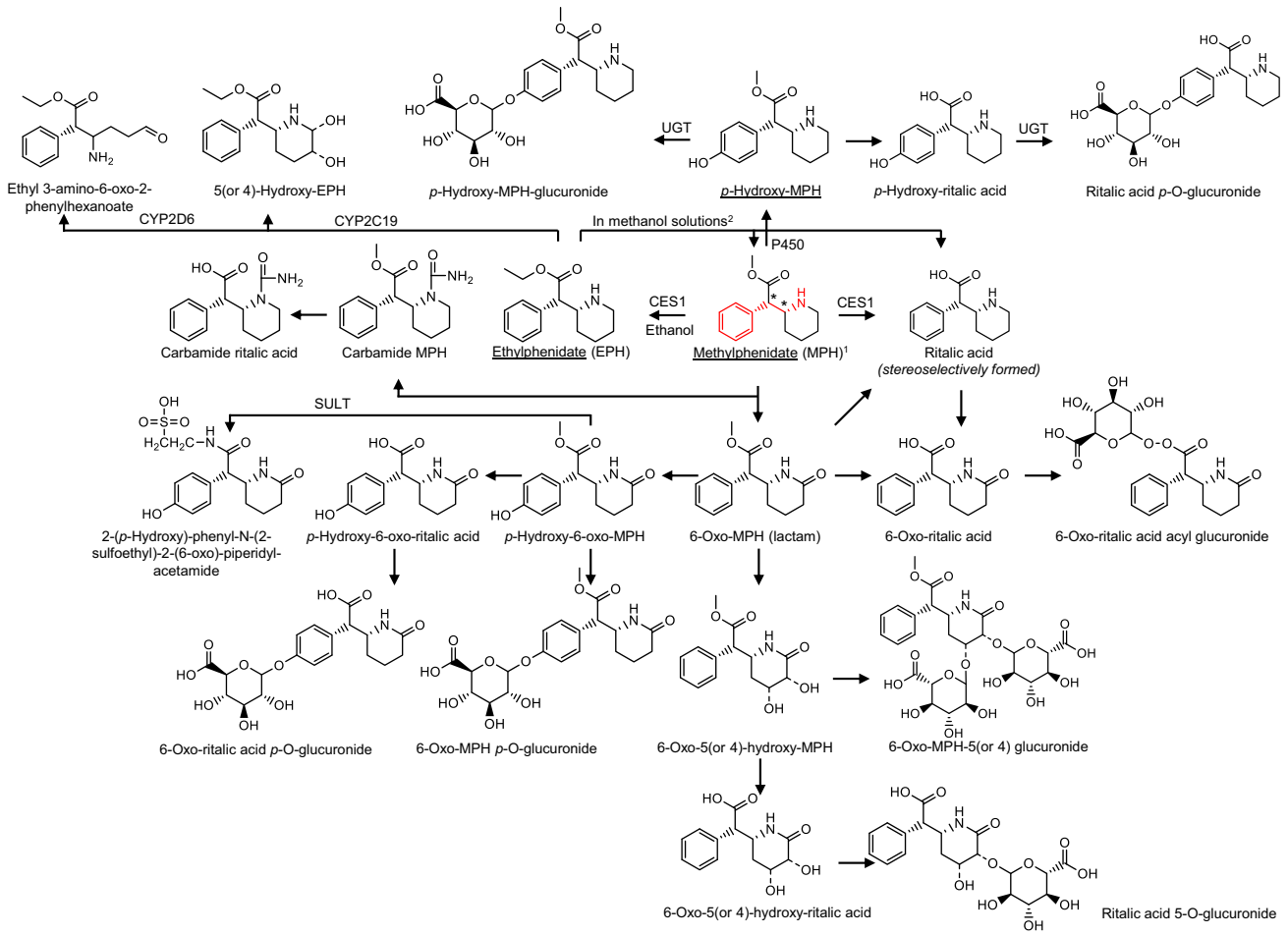

Fig. 1 Metabolomics of methylphenidate (MPH). The asymmetric carbon atoms are shown with*. [1]*d*-(2*R*, 2′*R*)-*threo*-methylphenidate (MPH) or *l*-(2*S*, 2′*S*)-*threo*-methylphenidate. [2]MPH is enzymatically and non-enzymatically formed from ethylphenidate (EPH). The common pharmacophore to dopamine, noradrenaline and amphetamine is depicted in red. Pharmacologically active compounds are underlined. *UGT* uridine diphosphate-glucuronosyltransferase, *SULT* sulfotransferase, *hCES* human carboxylesterase, *CYP* cytochrome P450

racemates exhibit similar hypertensive effects and lethal risk [5, 6]. Accordingly, the *dl-erythro* racemate was removed from pharmaceutical products to improve the therapeutic index. Nowadays, only the racemic mixture of *d-threo* (R, R)-MPH (or dexmethylphenidate) and *l-threo* (S, S)-MPH is being used [7]. The *d-threo*-enantiomer is much more active than *l-threo*-enantiomer and therefore, in some countries, pure formulations of *d-threo*-enantiomer have been used [4, 6, 8].

MPH is an indirect adrenomimetic since does not directly activate adrenoreceptors. Indeed, it inhibits the reuptake (i.e., competing for the uptake binding site) of monoamines such as dopamine and noradrenaline with a negligible effect on the 5-HT levels [1]. Inhibition of reuptake is achieved since MPH is substrate of dopamine (DAT), noradrenalin (NAT) and 5-HT (SERT) transporters [9]. Stimulation of the non-vesicular release and inhibition of monoamine oxidase A activity have also been reported, but seems to represent minor contributions [10–12]. Taking altogether MPH prolongs postsynaptic and presynaptic receptor interactions. Since it stimulates dopaminergic neurotransmission, addictive potential is likely but seems to lower than other amphetamines and cocaine due to slower brain clearance [13, 14].

MPH has been illicitly abused during exam periods to increase academic performance [15] and fatalities have been reported [16, 17]. Due to superimposed mechanism of action, pharmacodynamic interactions with other psychotropic drugs are likely [18].

Metabolomics is a relatively new field of “omics” technology (e.g., other approaches include genomics, transcriptomics, and proteomics) that is primarily concerned with the “global” quantitative and qualitative characterization of small molecules (i.e., the metabolome) at specific time points such as xenobiotics and their metabolites [19]. The focus of this manuscript is to perform a complete review of MPH metabolism. Indeed, although the use of MPH is frequent, it still poses a number of

unsolved questions, including its pharmacokinetic characteristics that may affect pharmacological and toxicological effects especially if ethanol is co-consumed since a new psychostimulant compound ethylphenidate (EPH) is formed by transesterification. EPH exhibits similar affinity for DAT, but less for NAT in comparison to MPH [20]. Further comprehension can be achieved by fully understanding the metabolomics of MPH and EPH.

2 Methodology

An exhaustive English literature search was carried out. Briefly, MPH and EPH known related metabolizing enzymes and metabolites were searched in PubMed (U.S. National Library of Medicine) without a limiting period. Furthermore, the retrieved journal articles as well as books were reviewed for possible additional publications related to human and non-human in vivo, in vitro and ex vivo studies on metabolomics of MPH and EPH.

3 Absorption, Distribution and Excretion

MPH is reported to be rapidly and completely absorbed from the intestine after oral administration with time to peak plasma concentrations (t_{max}) of ~ 1–3 h [21, 22]. It is a short-acting stimulant with a duration of action of ~ 1–4 h and an age-dependent pharmacokinetic half-life of ~ 2.5 h in children and ~ 3.5 h in adults [8, 21, 23]. Since it has a low degree of protein binding and a high lipid solubility, it its rapidly distributed and relatively large amounts of MPH may cross blood brain barrier [24]. In addition, because of the lipophilicity of MPH, the brain:plasma ratio in rats is 3:4 [25]. Different isomers have profoundly different tissue distributions [26].

The bioavailability of MPH ranges from 11 to 53 % in children [27]. After oral administration, 50 and 90 % of the [^{14}C]methylphenidate dose is excreted in urine by 8 and 48 h, respectively, and fecal elimination to 24 and 48 h accounted for 1.5 and 3.3 % of the radioactivity, respectively [25]. In urine, MPH is excreted 60–80 % as ritalinic acid and 5–12 % as 6-oxo-ritalic acid [28]. Less than 1 % is excreted as unchanged drug [29].

4 Metabolism of Methylphenidate

The metabolism of MPH has been described as extensive and stereoselective (Fig. 1) [29, 30]. The *threo* enantiomers are predominantly and rapidly hydrolyzed in the ester group via the endoplasmic reticulum human carboxylesterase 1 (CES1A1; a serine esterase) to the deesterified pharmacologically inactive metabolite *d*- or *l*-*threo*-ritalinic acid [2(or α)-phenyl-2(or α)-(2-piperidyl) acetic acid], which has a half-life of 3–4 h [8]. It is well recognized that the CES1A1 (highly expressed in liver, intestine, placenta and brain) exhibits six times higher enantioselectivity, preferring *l*-*threo*-enantiomer over *d*-*threo*-enantiomer, which logically has longer half-life [31–33]. Indeed, a gradual alteration in the plasma *d*/*l*-*threo*-MPH ratio over time is registered, and after 1.5 h plasma concentrations of *d*-*threo*-enantiomer are much higher than those of *l*-*threo*-enantiomer [21]. Polymorphisms in the human *CES1* gene that codifies to a much less active enzyme were already described [34]. This can lead to clinically significant alterations in pharmacokinetics by reducing MPH metabolism. Nevertheless, due to the low frequency of these variants, the effective impact at the population level needs further clarification. Aripiprazole, perphenazine, thioridazine and fluoxetine proved to be potent CES1A1 inhibitors, increasing the plasma concentrations of MPH [34, 35]. Therefore, drug interactions with MPH are likely to be mediated via CES1A1 inhibition due to concomitant drug therapies [35]. Taking altogether, lower MPH doses may be required in certain individuals and therapeutic drug monitoring of MPH should consider chiral chromatographic methods for the analysis of *d*-MPH.

Cytochrome-P450 (CYP) oxidation of the aromatic ring and further conjugation with glucuronic acid or deesterification represent minor pathways (< 2 %) of MPH metabolism [21]. *p*-Hydroxy-MPH, *p*-hydroxy-MPH-glucuronide, *p*-hydroxy-ritalic acid and ritalic acid *p*-*O*-glucuronide were already described [21]. Carbamide-MPH and carbamide-ritalic acid were described by some authors, but their toxicological relevance is unknown [25, 36]. *p*-Hydroxylation of MPH (but not ritalinic acid) suggests that this metabolite may play a role in the pharmacology of MPH [37].

Oxidative metabolism of MPH also occurs at the 6 position of the piperidine ring to yield the 6-oxo-MPH (lactam), which is also pharmacologically inactive [28, 37]. 6-Oxo-MPH can be further deesterified to 6-oxo-ritalic acid and then conjugated with glucuronic acid to form 6-oxo-ritalic acid acyl glucuronide or undergone to *p*-hydroxylation of the aromatic ring to *p*-hydroxy-6-oxo-MPH. This last metabolite can be then conjugated with sulfate to 2-(*p*-hydroxy)-phenyl-*N*-(2-sulfoethyl)-2-(6-oxo)-piperidyl-acetamide or with glucuronic acid to 6-oxo-MPH *p*-*O*-glucuronide. Alternatively, it may be deesterified to *p*-hydroxy-6-oxo-ritalic acid and further conjugated to 6-oxo-ritalic acid *p*-*O*-glucuronide [36]. Hydroxylated metabolites of the 6-Oxo-MPH piperidine ring at the 4 and 5 positions and subsequent conjugates with glucuronic acid were also described [21, 25].

Interestingly, some studies have demonstrated sex differences in the responsiveness to MPH, namely an

increased sensitivity of females to its stimulatory effects due to the achieved higher brain concentrations, particularly the active *d*-enantiomer [38]. Further studies are needed to access the contribution of metabolism for the registered differences.

5 Metabolism of Ethylphenidate

Similarly to cocaine that forms cocaethylene after ethanol consumption (Fig. 1) [39], CES1A1 also enantioselectively transesterifies *l*-MPH with ethanol to preferentially yield *l*-EPH [40]. Unlike cocaethylene, which has a longer half-life than cocaine, the half-life of *d*/*l*-EPH is shorter than that of the *d*/*l*-MPH [41]. The *d*-EPH is much more active than *l*-EPH and selectively targets the DAT, whereas *d*-MPH exhibits equipotent actions at DAT and NAT [41]. Due to its stimulant effects, EPH is being abused (racemic mixture) and fatalities were recently reported [42]. It is also believed by young people that the combination of MPH and ethanol allows that higher doses of ethanol may be consumed prolonging rave time [43].

As previously reported, concentrations of EPH in blood and plasma are very unstable due to hydrolysis by blood esterases [44]. Storage at temperatures lower than 4 °C and tubes containing sodium fluoride may be used to increase stability [45], but further stability studies are needed. Ritanilic acid is also a metabolite of EPH, and therefore not specific to distinguish between MPH and EPH consumption. Besides MPH itself, several other metabolites could be used as alternative to ritalinic acid such as *p*-hydroxy-MPH and 6-oxo-MPH (Fig. 1). Unexpectedly, standard solutions of EPH prepared in methanol and storage at − 20 °C in the dark also produce ritalinic acid and MPH by hydrolysis and *trans*-esterification, respectively [46]. Recently, CYP2C19 showed to be most active recombinant enzyme involved in the formation of EPH metabolites [46]. Authors proposed that two primary mono-hydroxylated metabolites of the piperidine ring in 4 and 5 positions [i.e., 5(or 4)-hydroxy-EPH] are produced by CYP2C19. EPH also underwent ring opening forming ethyl 3-amino-6-oxo-2-phenylhexanoate due to hydroxylation of the alpha carbon to the nitrogen atom and subsequent oxidation to the respective aliphatic aldehyde; this reaction is catalyzed by CYP2C19 and CYP2D6 [46]. Hydroxylation in the aromatic ring was not yet registered.

6 Conclusions and Future Perspectives

ADHD is a highly prevalent childhood neurodevelopmental disease that may compromise academic, family, and social relationships [47]. The cardinal symptoms include inattentiveness, hyperactivity, and impulsivity, which may be present together or individually. MPH is nowadays the most commonly and increasingly prescribed psychostimulant used in children diagnosed with ADHD due its calming effect in hyperactive children. Pharmacodynamics is mainly due to three mechanisms: inhibition of noradrenaline and dopamine reuptake, facilitation of their release into the synaptic cleft, and inhibition of monoamine oxidase activity [10, 11].

In this work, metabolomics of MPH was fully reviewed. Ritalinic acid is the main urinary metabolite. In addition, minor pathways involving aromatic hydroxylation, microsomal oxidation and conjugation have also been reported to form the *p*-hydroxy-, oxo- and conjugated metabolites, respectively. To better understand clinical effects, pharmacokinetics should be explored to find for active and inactive metabolites. Indeed, the metabolic profile, namely polymorphisms in genes encoding enzymes involved in xenobiotic metabolism, may influence both drug efficacy and toxicity. Moreover, identification of metabolites is needed during drug development and for clinical and forensic toxicology, where specific metabolites are used to confirm xenobiotic exposure [48]. Regarding MPH, further knowledge regarding the pharmacological profiles of the enantiomers is needed for a more efficacious therapeutic drug monitoring. Finally, studies are required to study the contribution of metabolites for MPH pharmacodynamics.

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Compliance with Ethical Standards

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